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Use of AQUI-S®20E and BENZOAK® to Sedate Sunshine Bass, Blue Catfish, and Nile Tilapia to Handleable

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Sedatives are chemicals or physical agents that—with increasing treatment concentration and duration—first calm an animal and then cause successive loss of mobility, equilibrium, consciousness, and reflex action. Fisheries professionals routinely sedate fish for a variety of purposes, including collection of samples or morphometric data, implantation of tags or tracking devices, and transport. Sedating fish before handling can minimize stress and physical injury to the fish and also help protect the handler. Ideally, a fish sedative is safe, effective, easy to administer, and inexpensive. Also, it is desirable that the sedative have no withdrawal period so that treated fish can be released into the wild immediately after recovery from sedation.

Currently, only tricaine methanesulfonate (tricaine or MS222) is approved by the U.S. Food and Drug Administration (FDA) for the temporary immobilization of fish and other aquatic, cold-blooded animals. The two tricaine products available in the U.S. are TRICAINE-S (Western Chemical, Inc., Ferndale, Washington USA) and FINQUEL (Argent Laboratories, Redmond, Washington USA). Both are effective and widely used by fisheries professionals; however, a 21-day withdrawal period is required for fish entering the human food chain through stocking or slaughter. For many field applications, holding fish for 21 days postsedation is not practical and seriously compromises management or research activities.

In the U.S., efforts are underway to obtain FDA approval of AQUI-S®20E (10% eugenol; AQUI-S New Zealand, Ltd., Lower Hutt, New Zealand) and BENZOAK® (20% benzocaine; Europharma USA, Victoria, Minnesota USA) as immediate-release fish sedatives. Considerable research has shown that eugenol and benzocaine are efficacious for sedating freshwater and saltwater fishes to handleable (e.g., Trushenski et al. 2012a, 2012b, 2012c). However, FDA requires data that demonstrate a product is effective in its final formulation at its proposed lowest efficacious dose. To help obtain FDA approval of AQUI-S®20E and BENZOAK® for use on all freshwater-reared, warmwater finfish, we conducted three independent trials to evaluate the efficacy of these two sedatives for sedating adult sunshine bass (SSB) female *Morone chrysops* × male *M. saxatilis*, subadult blue catfish (BCF) *Ictalurus furcatus*, and juvenile Nile tilapia (NTLP) *Oreochromis niloticus* to handleable.

Methods

The trials were conducted in September 2011 at the Southern Illinois University (SIU) aquaculture facility in Carbondale, Illinois USA. In each trial, fish were sedated to handleable at 60 mg per L eugenol (600 mg per L AQUI-S®20E), 150 mg per L benzocaine (750 mg per L BENZOAK®), or 150 mg per L tricaine (active control). We tested 60 mg per L eugenol and 150 mg per L benzocaine because these are the lowest efficacious doses that will be proposed for warmwater finfish on the respective product labels. A fish was determined to be handleable when it lost equilibrium and the ability to swim, could easily be caught by and held in hand, and did not struggle while being measured for length.

Ninety fish were used in each trial, i.e., for each sedative, 30 fish were individually sedated under static conditions. Sedative solutions were prepared in bulk (e.g., 40 gal), and aliquots of these solutions were used to fill individual sedation containers. The contents of each sedation container were discarded after one fish had been sedated. When a fish was determined to be handleable, it was removed from the sedative solution, measured for length, and transferred to a container of fresh, flowing water. A fish was determined to be recovered when it regained equilibrium, resumed normal swimming behavior, and avoided obstacles (e.g., a net handle) placed in its path. Times to handleable and recovery were determined for each fish, and general fish behavior was assessed during sedation and recovery. Following recovery, fish were placed in a holding tank plumbed with fresh, flowing water and monitored for survival for 24 h.

In each trial, water temperature and dissolved oxygen (DO) concentration were measured in each sedation container before a fish was placed into the solution. Sedative solution samples were collected from 30 randomly selected sedation containers (10 per sedative) and analyzed by spectrophotometry to verify concentrations of either eugenol or benzocaine. Water hardness, alkalinity, and pH were measured once in the source water used in each trial.

Results and Discussion

Across all three trials, all fish became handleable within 4.9 min and recovered from handleable within 14.2 min (Table 1); there were no postsedation mortalities. Also, some fish

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exhibited "piping" behavior at the surface shortly after being placed into a sedative solution; however, this behavior ceased after a few seconds as the fish became sedated. Behavior during recovery was usually characterized as normal; however, a few SSB were observed "spitting" water from their mouths while piping at surface of the water, and one NTLP was observed "whirling." Mean water temperature and DO concentration ranged from 24.0 to 25.7°C and 7.6 to 8.0 mg per L, respectively (Table 2). Source water hardness, alkalinity, and pH were within ranges suitable for rearing healthy warmwater finfish (Table 2). Mean analytically verified concentrations of eugenol and benzocaine were within ±16.1% of their respective target concentrations (Table 3).

Based on these results, we concluded that AQUI-S®20E and BENZOAK® were effective in sedating adult SSB, subadult BCF, and juvenile NTLP to handleable under the conditions tested. Although many factors (e.g., sedative concentration, fish life stage, water temperature) can influence time to sedation and recovery, we speculate that other warmwater finfish tested under similar conditions will become sedated and recover in times similar to those observed in our trials. Results from these trials were submitted to and accepted by FDA as providing sufficient evidence of efficacy for AQUI-S®20E and BENZOAK®.

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References

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Table 1. Times to handleable and recovery.

Trial	Species	Mean time (minutes) to handleable (range)			Mean time (minutes) to recovery (range)		
		AQUI-S®20E	BENZOAK®	Tricaine	AQUI-S®20E	BENZOAK®	Tricaine
1	Sunshine bass	0.9 (0.6 – 1.4)	0.9 (0.6 – 1.9)	1.5 (1.0 - 2.4)	5.2 (3.5 – 7.6)	4.7 (2.3 – 14.2)	4.4 (2.0 – 8.1)
2	Blue catfish	1.4 (0.8 – 2.1)	1.6 (0.8 – 4.9)	1.8 (0.8 – 3.6)	5.4 (1.5 – 8.7)	6.2 (4.3 – 10.0)	3.8 (2.0 – 6.2)
3	Nile tilapia	1.8 (0.9 – 3.2)	1.1 (0.5 – 2.5)	2.3 (1.4 – 3.8)	3.8 (2.4 – 5.8)	3.0 (1.4 – 8.9)	3.1 (1.3 – 4.3)

Table 2. Water quality parameters measured.

Trial	Fish		Mean water quality parameters				
	Species	Mean ± SD total length (cm)	Temperature (°C)	Dissolved oxygen concentration (mg per L)	Hardness (mg per L as $CaCO_3$)	Alkalinity (mg per L as $CaCO_3$)	pH
1	Sunshine bass	38.2 ± 1.9	24.3	8.0	142	141	7.9
2	Blue catfish	22.0 ± 2.7	24.0	7.6	138	124	7.9
3	Nile tilapia	10.8 ± 0.8	25.7	7.7	58	599	8.9

Table 3. Eugenol and benzocaine concentrations administered.

Trial	Species	Mean concentration administered (% difference from target)	
		Eugenol (mg per L)	Benzocaine (mg per L)
1	Sunshine bass	59.2 (-1.3)	160.0 (+6.7)
2	Blue catfish	59.7 (-0.5)	146.1 (-2.6)
3	Nile tilapia	57.3 (-4.5)	125.9 (-16.1)